Travel-associated acquisition of hepatitis C virus infection in patients receiving haemodialysis

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Abstract

Background. It has been proposed that hepatitis C virus (HCV)-infected patients with end-stage renal disease undergoing maintenance haemodialysis may lack HCV antibody (anti-HCV) despite chronic HCV viraemia. This carries important implications for the design of surveillance policies.

Methods. To characterize the prevalence of antibody-negative/RNA-positive HCV infection, patients attending seven haemodialysis units underwent anti-HCV testing using a third-generation assay and HCV RNA testing using real-time PCR.

Results. At screening, anti-HCV prevalence was 12/360 (3.3%; 95% CI 1.7–5.8%); 7/12 (58.3%) anti-HCV positive samples were HCV RNA positive. Among anti-HCV-negative samples, 2/348 (0.6%; 95% CI 0.2–2.1%) tested HCV RNA positive (genotype 1a). Retrospective testing of stored sera dated the infections to a period of holiday in the Indian subcontinent. The two infections were unrelated by HCV-NS5B sequencing. Only one of the two newly infected persons showed raised transaminases. Both developed anti-HCV within 8–13 weeks of follow-up. Prospective surveillance of travellers to resource-limited countries returning to the units showed a HCV incidence of 4/153 travel episodes (2.6%; 95% CI 0.7–6.6%) among 131 persons (3.1%; 95% CI 0.8–7.6%).

Conclusions. Among haemodialysis patients in the United Kingdom, antibody-negative/RNA-positive HCV status is associated with newly acquired infection, rather than lack of antibody responses in chronic HCV infection. There is a significant risk of HCV infection associated with travel to resource-limited countries. Given that transaminase levels may be normal, HCV RNA testing is recommended in patients re-entering a dialysis unit following haemodialysis in settings where suboptimal infection control policies pose a risk of exposure to blood-borne viruses.

Keywords: haemodialysis; hepatitis C virus; HCV antibody; HCV RNA; seroconversion; seroprevalence

Introduction

Patients with end-stage renal disease (ESRD) undergoing maintenance haemodialysis show an increased risk for hepatitis C virus (HCV) infection relative to the general population, with seroprevalence rates across dialysis centres in Western Europe ranging from 3% in the United Kingdom to 30% in France [1]. The increased prevalence of infection reflects the presence of common risk factors for HCV acquisition, including a history of drug abuse preceding ESRD [2] and transfusion or transplantation before 1994 [3], but also susceptibility to nosocomial transmission during dialysis [2,4]. A prompt diagnosis is important for appropriate patient management and prevention of further transmission [5]. There is evidence that improved infection control policies have reduced the risk of nosocomial HCV transmission in many resource-rich countries [1].

Current guidelines recommend that ESRD patients on haemodialysis undergo routine surveillance for infection with blood-borne viruses, including antibody testing for HCV [6]. Previous studies have documented that HCV viraemia can occur in the absence of detectable anti-HCV in ESRD patients on haemodialysis, a phenomenon sometimes described as ‘cryptic’ HCV infection [7–14]. Factors postulated to play a role in reducing the antibody responses to HCV include the...
immune suppressive effect of chronic uraemia, presence of high pro-inflammatory cytokine levels and concomitant occurrence of diabetes [12,13]. Based on these findings, several investigators have recommended HCV RNA testing for the routine surveillance of dialysis patients with undetectable anti-HCV [10,12,13]. This recommendation is not uniformly applied and practice varies considerably.

The detection of HCV antibodies in infected persons is dependant on the sensitivity of the anti-HCV assay used for screening. Thus, the prevalence of antibody negative/RNA positive-HCV infection may have been overestimated in some of the older studies that employed second-generation antibody tests [15]. Detection of HCV RNA in the absence of may also reflect the prolonged window period before seroconversion that characterizes acute HCV infection. Even with current third-generation assays, the window period can be prolonged relative to the timing of infection and onset of viraemia.

The aim of this study was to determine the prevalence of antibody-negative/RNA positive HCV infection in a large population of ESRD patients receiving maintenance haemodialysis and undergoing routine surveillance by a third-generation anti-HCV assay, with the aim of informing monitoring protocols. Having detected a risk of HCV infection during travel to resource-limited countries, a further aim was to quantify this risk by conducting intensive HCV RNA monitoring in returning travellers.

Materials and methods

The study population comprised 360 patients undergoing haemodialysis at seven dialysis units in London. With the patients’ informed consent, freshly drawn serum samples underwent anti-HCV testing by the VITROS Eci HCV assay (Ortho Clinical Diagnostics, UK), which is based on the three recombinant HCV antigens c22-3, c200 and NS-5. The samples also underwent testing for the presence of HCV RNA using a quantitative real-time PCR with a lower limit of detection of 23 IU/ml (HCV RealTime PCR, Celera, United States). Genotyping was done by the Versant HCV Genotype (Line Probe Assay) (Bayer Healthcare Diagnostic Division, UK). Samples that tested positive for anti-HCV and negative for HCV RNA underwent confirmation of anti-HCV status by the Recombinant Immunoblot Assay (RIBA) (Chiron RIBA HCV 3.0 SIA, Ortho Clinical Diagnostics, USA).

For sequencing of the HCV-NS5B region, nucleic acid from 150 μl of serum was extracted using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics Limited, UK) according to the manufacturer’s instructions. HCV RNA was reverse transcribed into cDNA using random hexamers (Amersham Pharmacia Biotech, UK) and Murine Molony leukaemia virus reverse transcriptase (Invitrogen, UK). Primary amplification of the NS5B region of the HCV genome was carried out using primers 1203 and 1204. Secondary PCR products were purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech) and sequenced using the GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, UK) on a CEQ8000 sequencer (Beckman Coulter, UK). Sequences were aligned and compared to a background of samples of known subtype using the CLUSTAL V algorithm in the MEGALIGN program of the LASERGENE package (DNASTAR Inc, Madison, WI, USA). Once the subtype had been determined the sequences were compared to other samples of the same subtype derived from all sequences collected at the Virus Reference Department of the Health Protection Agency in 2005. All samples were stored at −70°C.

In the statistical analysis, 95% confidence intervals (CI) were calculated using the exact Wilson method. Factors associated with the presence of anti-HCV were investigated using logistic regression models. Only a limited number of demographic and clinical parameters collected routinely in clinical practice were available for analysis (HCV status, gender, age and duration of haemodialysis).

Results

Prevalence of anti-HCV-positive infection

The demographic characteristics of the study population are summarized in Table 1. Overall, anti-HCV seroprevalence was 12/360 (3.3%; 95% CI 1.7–5.8%). Among patients with detectable anti-HCV, 7/12 had detectable HCV RNA in serum, with a median viral load of 349 000 IU/ml (range 6360 to 2.3 million IU/ml). HCV genotypes included 1 (n = 2), 1b (n = 1), 3a (n = 2) and 4/4e (n = 2). The anti-HCV status of those who tested HCV RNA negative was confirmed by RIBA. The association between anti-HCV status, age, gender and duration of haemodialysis was investigated using univariate (unadjusted) and multivariate (adjusted) logistic regression models. There was a trend for anti-HCV-positive persons to be younger than anti-HCV-negative persons, with a decrease in the odds of having HCV by 25% for every 10 years increase in age (odds ratio = 0.075; 95% CI 0.54–1.04; P = 0.09). There was no effect of gender or length of dialysis.

Table 1. Demographic characteristics of haemodialysis population screened for hepatitis C (HCV) infection by HCV antibody (anti-HCV) and HCV RNA testing

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole population N (%)</th>
<th>Anti-HCV-positive N (%)</th>
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</thead>
<tbody>
<tr>
<td>Total</td>
<td>360 (100%)</td>
<td>12 (3.3%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>213 (59%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Females</td>
<td>147 (41%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Median age at start of dialysis years (range)</td>
<td>59 (10–90)</td>
<td>45 (31–65)</td>
</tr>
<tr>
<td>Median duration of haemodialysis, years (range)</td>
<td>3 (0–37 years)</td>
<td>4 (2–23 years)</td>
</tr>
</tbody>
</table>
Prevalence of anti-HCV negative infection

The 348 anti-HCV negative persons underwent testing for HCV RNA using a real-time HCV PCR assay with a lower limit of detection of 23 IU/ml. Of these, 2/348 (0.6%; 95% CI 0.2–2.1%) tested positive for HCV RNA, with a viral load of 3.9 million IU/ml and 1.7 million IU/ml, respectively. These findings were confirmed by repeated testing of the same sample and in a subsequent sample. On the date of the first positive HCV RNA results, liver function tests showed the following abnormal results: Patient 1: alkaline phosphatase (ALP) 167 U/l [normal range 42–128], aspartate aminotransferase (AST) 174 U/l [normal range 5–40], alanine aminotransferase (ALT) 226 U/l [normal range 5–40]; Patient 2: ALP 172 U/l. The two patients had been receiving dialysis for 8 and 2.4 years, respectively. During the 2 months before the first positive HCV RNA result, they had visited the Indian subcontinent (India and Bangladesh, respectively) where they had received dialysis. Retrospective testing was undertaken of stored samples collected up to the time immediately before travel. A total of five and two samples were tested in the two patients, respectively and all were HCV RNA negative. Prospective testing demonstrated anti-HCV seroconversion 8 and 13 weeks, respectively, after the first HCV RNA positive result. Both patients were infected with HCV genotype 1a. Phylogenetic analysis of the HCV NS5B region demonstrated that the two infections were not related to each other (Figure 1).

Intensive surveillance of patients returning from a period of dialysis abroad

Following the detection of the two cases of acute HCV infection, a pilot scheme of intensive surveillance was adopted for all patients returning to the units after having received dialysis in resource-limited settings. The patients underwent weekly HCV RNA testing for the first 8 weeks after return, together with the adoption of segregation facilities for dialysis. In the period between May 2005 and October 2006, four additional cases of newly acquired HCV infection with genotypes 1 (n = 2), 1b (n = 1) and 3b (n = 1) were identified in persons returning from Singapore, Pakistan, Slovakia and Bangladesh, respectively. This resulted in an incidence of four cases per 153 episodes of travel (2.6%; 95% CI 0.7–6.6%) among 131 patients (3.1%; 95% CI 0.8–7.6%).

Discussion

In this study we used a third-generation antibody assay and a highly sensitive real-time PCR assay to determine the prevalence of antibody-negative/RNA-positive HCV infection in a large unselected cohort of patients receiving maintenance haemodialysis. Antibody-negative/RNA-positive HCV infection was uncommon in this cohort, with an overall prevalence of 0.6%, and this was entirely accounted for by two persons with newly acquired infection who subsequently showed anti-HCV seroconversion.

Table 2. Univariate and multivariate analysis of factors associated with hepatitis C (HCV) antibody (anti-HCV) positive status in a haemodialysis population including 360 persons of whom 12 were anti-HCV positive

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Ref</td>
<td>–</td>
</tr>
<tr>
<td>Female</td>
<td>0.80</td>
<td>0.26, 2.43</td>
</tr>
<tr>
<td>Duration of dialysis</td>
<td>1.02</td>
<td>0.95, 1.11</td>
</tr>
<tr>
<td>Age at start of dialysis</td>
<td>0.77</td>
<td>0.58, 1.03</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval.
Previous studies based on the use of third-generation anti-HCV assays reported rates of antibody negative/RNA positive HCV infection in haemodialysis patients ranging from 0.4% in the Netherlands [10] and 0.9% in Germany [11] to 9% in Israel [12] and 12% in Greece [14]. The overall prevalence of anti-HCV positive infection appears to follow similar patterns, ranging from 3.5% in the Netherlands [10] to 36% in Greece [14]. We found that the prevalence of anti-HCV positive infection was 3.3%, and similar to that observed in other Northern European countries [1]. Time on dialysis has been identified as a risk factor for HCV infection, reflecting both the cumulative risk of infection over time and the immunosuppressive effects of prolonged dialysis [13]. Univariable and multivariable analysis did not identify predictors of anti-HCV positivity among the limited number of factors analysed in this study (gender, age, duration of dialysis). This is likely to result from the small number of observations. A trend was detected however for a higher risk of anti-HCV positivity among younger patients, suggesting a possible impact of HCV on ESRD progression.

Prospective testing of the two patients with antibody-negative/RNA-positive HCV infection demonstrated that they were in the window period for anti-HCV seroconversion that characterizes acute HCV infection. Both seroconverted during follow-up, but remained viraemic for 2–3 months prior to the appearance of anti-HCV. The source of the infection was not determined conclusively. However, both patients were repeatedly HCV RNA negative prior to an extended period of holiday in the Indian subcontinent, and HCV RNA positive immediately on return, indicating that the infection had most likely been acquired during the holiday. With the exclusion of haemodialysis, no other risk factors for infection were identified. Dialysis abroad has been identified as a risk factor for HCV infection among ESRD patients [3]. It should be noted that only one of the two patients had abnormal transaminase levels, indicating that liver function tests cannot be used reliably for screening purposes in this setting. By adopting HCV RNA testing for the intensive surveillance of patients returning from a period of dialysis in resource-poor settings, we detected four additional cases of newly acquired infection. Thus the risk of HCV infection is significant in returning travellers.

Strict infection control procedures play a crucial role in reducing the risk of infection with blood-borne viruses in the haemodialysis setting. Surveillance protocols vary however. Findings from this study show that antibody-negative/RNA-positive HCV status is associated with newly acquired infection among haemodialysis patients in the United Kingdom, rather than the lack of an antibody response in persons with chronic infection. Thus, routine surveillance by HCV RNA testing is not warranted in this population. There is a significant risk of HCV infection associated with travel to resource-limited countries however. Given that transaminase levels may be normal despite a recent HCV infection, returning travellers from resource-limited settings re-admitted to a dialysis unit should undergo screening and intensive surveillance by HCV RNA testing, to account for the prolonged window period prior to anti-HCV seroconversion. Prompt recognition of newly acquired infection allows appropriate management of the infected patient and the adoption of measures that limit the risk of further transmission. Before travel, patients should be made aware that the risk of infection is substantial.

Conflict of interest statement. None declared.

References

6. Good Practice Guidelines for Renal Dialysis/Transplantation Units: Prevention and Control of Blood-borne Virus Infection; Department of Health (UK): Recommendations of a working group convened by the Public Health Laboratory Service (PHLS) on behalf of the Department of Health


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